

ENDOCRINE GLANDS AND BILATERAL SYMMETRY: OBSERVATIONS UPON FORELIMB ERUPTION IN FROG LARVÆ UNDER TREATMENT WITH THYROID AND THYMUS EXTRACTS.

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INTRODUCTION.

A conspicuous feature of thyroid-accelerated metamorphosis in frog larvæ is the eruption of the forelimbs. Before these become visible externally they are located beneath the skin in the gill chamber. For some years the writer has been aware of the fact that the left forelimb is protruded before the right forelimb, sometimes by as much as several days. This condition was recorded in a previous paper (Jordan and Speidel, '23) and has probably also been noted by other workers in the field of amphibian metamorphosis. In half-grown bullfrog and green frog tadpoles no exceptions to this were seen; *i.e.*, no case appeared of precedent right forelimb eruption in thyroid-treated tadpoles. It was, therefore, of some interest to find in a jar of seven thymus-treated tadpoles one animal in which the right forelimb appeared two weeks before the left. Furthermore, in two other animals in this lot the amount of skin degeneration in the right forelimb region was definitely farther advanced than that on the left side, a condition indicating probable prior right forelimb eruption. Death ensued, however, before the appearance of either forelimb. Two of the other four animals in this jar put out the left forelimb first, and in one the amount of skin degeneration on the left side plainly foreshadowed the prior eruption of the limb of that side. The remaining animal put out both limbs over night. In this jar of seven thymus-treated animals, therefore, the ratio of "right-handed" to "left-handed" animals is 1 : 1.¹

The question suggests itself as to whether the endocrine secre-

¹ The terms "right-handed" and "left-handed" are used to denote merely prior right forelimb eruption or prior left forelimb eruption, respectively.

tions represented by the thyroid and thymus extracts actually affect in a differential manner the bilateral symmetry of the developing animals. Further experiments were set under way in an attempt to analyze the factors controlling variation in forelimb eruption.

There is a difference of opinion as to the condition in normal frog larvæ. Barfurth ('87) in Europe finds in the case of *Rana fusca* that 80 per cent. put out the right forelimb first, the left almost always following in from two to eight hours. On the other hand, Gudernatsch ('14), using *Rana temporaria* and *Rana esculenta*, states that he has always found the reverse to be the case; *i.e.*, in about 80 per cent. the left forelimb erupts first.² Dickerson ('20) observes that in normal bullfrog and green frog metamorphosis the left forelimb is usually put out first. My own observations upon the normal tadpoles of *Rana sylvatica*, *Rana clamata*, *Rana catesbeiana*, and *Hyla crucifer* lead me to agree with Gudernatsch and Dickerson that in a definite majority of cases the left limb is the first to erupt.

MATERIAL, EXPERIMENTS AND OBSERVATIONS.

The material used includes about 800 tadpoles of *Hyla crucifer*, *Rana catesbeiana*, *Rana clamata*, and *Rana sylvatica*, and a few of *Rana cantabrigiensis* and *Rana pipiens*. The bullfrog and green frog tadpoles were collected at Charlottesville, Virginia, the others at Woods Hole, Massachusetts. Untreated animals were usually kept in aquaria containing pond water and weed. Administration of endocrine extract was accomplished by placing some of the extract in the water with the animals. Thyroid and thymus desiccated extracts were used.

The accompanying table indicates the relative frequency of left or right forelimb eruption, as it occurs under normal conditions, under thyroid treatment, and under thymus treatment.

² Gudernatsch makes this statement in a footnote referring to his experiment with thyroid-accelerated metamorphosis. It is probable, therefore, that he included his observations on thyroid-treated animals with those on normal animals in regard to forelimb eruption, not realizing that thyroid treatment affects forelimb eruption. Thyroid administration, as shown by this paper, markedly favors the prior eruption of the left forelimb. His percentage, therefore, is not correct, but is too high in favor of lefthandedness.

TABLE I.

In this table is given for each species of tadpole the number of individuals observed with prior left forelimb eruption as compared with the number of individuals observed with prior right forelimb eruption, under normal conditions, under thyroid treatment and under thymus treatment.³

	Normal-untreated.		Thyroid-treated.		Thymus-treated.	
	Prior Left.	Prior Right.	Prior Left.	Prior Right.	Prior Left.	Prior Right.
<i>Hyla crucifer</i>	43 (72%)	17 (28%)	25 (100%)	0	17 (50%)	17 (50%)
<i>Rana sylvatica</i>	42 (65%)	22 (35%)	19 (100%)	0		
<i>Rana catesbeiana</i> and <i>R. clamata</i>	3	0	85 (100%)	0	3 (50%)	3 (50%)
<i>Rana cantabrigiensis</i>			4 (100%)	0		
<i>Rana pipiens</i>			20 immature tadpoles, all died before either forelimb erupted			
Total, all species	88 (70%)	39 (30%)	133 (100%)	0	20 (50%)	20 (50%)

Under normal conditions a definite majority of tadpoles puts out the left forelimb first; in *Hyla* 72 per cent., in *Rana sylvatica* 65 per cent. The few observations upon untreated bullfrog and green frog tadpoles in the three-limb condition support Dickerson's observation that the left limb usually erupts first. Under thyroid treatment the left forelimb is invariably protruded first in all species studied, if those putting out forelimbs during the first forty hours of the treatment are excluded. Under thymus treatment apparently a 1 : 1 ratio is indicated. The experiments with *Hyla* afford the best comparative figures and may be taken as typical.

With thyroid treatment of half-grown tadpoles 100 per cent. put out the left forelimb first. A special experiment was tried in which 75 *Rana sylvatica* tadpoles were subjected to thyroid treatment, many of these at the time being on the verge of putting out the forelimbs. Among those animals putting out forelimbs within the first forty hours, seven righthanded ones were seen; thereafter all were lefthanded. It may be concluded that in animals protruding the forelimbs within this time, an original bias toward righthandedness may not be changed. In a similar experiment with 25 *Hyla* tadpoles that were also fairly close to

³ For reasons explained in the text all animals putting out forelimbs within the first two days of thyroid or thymus treatment are discarded, and do not figure in the table.

the time of forelimb eruption, one righthanded animal occurred during the first 24 hours: after that all were lefthanded. For this reason, in Table I. all animals putting out forelimbs during the first two days are omitted from the reckoning, since the original normal bias may not have been sufficiently influenced by the endocrine extract.

How long it takes for the thymus extract to affect an original bias is not known. Since it is probably not so powerful as the thyroid it may be that more than the first two days' results should be discarded. If the first two days are discarded the ratio is 17 : 17 as given in the table. If the results of the first three to seven days are discarded the ratio shifts progressively to favor righthandedness. Only a much larger number of animals under observation would give a trustworthy ratio. The figures given, however, are in all probability enough to show that the normal ratio has been affected and shifted in the direction of righthandedness.

INTERPRETATION AND DISCUSSION.

These observations leave no doubt that the bilateral symmetry of the developing frog tadpole, as indicated by the relative time of forelimb eruption, is influenced by thyroid extract; possibly also by thymus extract. The action of the thyroid extract will first be discussed. The blood carries the active thyroid principle to all parts of the body. It is inconceivable that the thyroid autacoid should have one effect on the tissues on the left side and another effect on exactly similar tissues on the right side. Therefore, the effect of the thyroid in changing the normal symmetrical development must be due to some original fundamental asymmetry of the body pattern.

The following findings are pertinent: The tadpole is conspicuously asymmetrical in respect to its respiratory apparatus. A spiracle, or outlet from the gill chamber, is present on the left side only (Fig. 1). This outlet drains both left and right gill chambers, these being connected by a canal across the mid-line. The forelimb is present in the gill chamber, its degree of development depending upon the general developmental state of the tadpole. Both in normal and thyroid-induced metamorphosis

the left limb is pushed through the spiracle, a variable amount of preliminary skin degeneration occurring. The right forelimb erupts through the skin only after the latter has undergone a certain amount of degeneration. This degeneration starts in the



FIG. 1.



FIG. 2.

FIG. 1. Ventral view of a tadpole of the tree frog (*Hyla crucifer*) showing well developed forelimbs (*j*) still imprisoned beneath the skin in the gill chambers (*r.g.c.* and *l.g.c.*). The position of the sinistral spiracle (*s*) is shown, which drains both gill chambers; also the canal (*c*) across the mid-line which connects right and left gill chambers. The dotted line below the forelimbs indicates the position of the partition separating the gill chambers from the abdominal cavity.

FIG. 2. Dorsal view of a thyroid-treated tadpole (*Hyla crucifer*) in typical three-limb stage after the eruption of the left forelimb. Collection and retention of air (*a*) in the right gill chamber causes bulging out of the skin and interferes with forelimb eruption on that side. Excluding the first two days, prior left forelimb eruption occurs invariably.

vicinity of the elbow, and the elbow is usually protruded first. Movements of the forelimb finally enable it to break completely through. In young thyroid-treated tadpoles, however, the forelimbs are little developed so that the elbow is not prominent at the surface. In these cases the hand or whole arm appears as a tiny white stump after the skin degeneration has proceeded far enough.⁴

The administration of thyroid extract to a half-grown tadpole obviously upsets the respiratory mechanism. More air is taken

⁴While this paper was in press, Helff ('24), at the Washington meeting of the American Society of Zoölogists, reported a series of experiments which show clearly that the opercular skin autolysis preceding forelimb eruption is brought about by substances given off by the adjacent atrophying gills.

in. But apparently the animal is not yet very well fitted for utilizing it properly. Small bubbles of air collect in the gill chambers and are not expelled, or expelled with difficulty. This is especially true of the right gill chamber which has no outlet except through the left spiracle (and, of course, the mouth). This proves to be the deciding factor. The left gill chamber is usually drained well enough except in very immature tadpoles, so that skin degeneration and forelimb eruption on that side are not interfered with. On the right side, however, the retention of air in the chamber causes bulging out of the skin (Fig. 2), and interferes to a greater or less degree with the normal eruption on that side, thus bringing about the typical prior left forelimb eruption. A tadpole in this stage does not present a normal posture when at rest, but floats with the right side somewhat elevated owing to the air in the right gill chamber. The difference between a thyroid-treated animal in this condition and either normal or thymus-treated animals may be seen by comparing Fig. 2 with Figs. 3 and 4. Occasionally, in very young

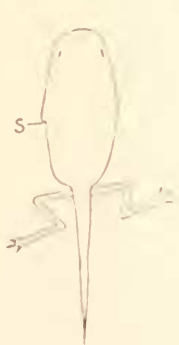


FIG. 3.



FIG. 4.

FIG. 3. Dorsal view of a normal tadpole (*Hyla crucifer*) before the eruption of either forelimb. The location of the spiracle (*s*) on the left side only, leads to a majority (70 per cent.) of prior left forelimb eruptions.

FIG. 4. Dorsal view of a thymus-treated tadpole (*Hyla crucifer*) showing prior right forelimb eruption. Either right or left forelimb may erupt first. In the region of the spiracle (*s*) may be seen an angular projection (*p*) caused by the pressure of the imprisoned left forelimb.

and immature tadpoles no forelimbs erupt with thyroid treatment, the animals dying in the two-limb stage. In these, bubbles of air may be seen in both gill chambers. The pulmonary

development of the animal is not adequate to the demands imposed upon it by thyroid treatment and death results. Coupled with this respiratory disturbance is also the condition of anemia already pointed out elsewhere (Jordan and Speidel, '23). The older the tadpole the more probable it is that both forelimbs will erupt. The pulmonary apparatus is presumably better developed, and the forelimbs are large enough so that limb movements aid both in expelling the air from the gill chamber and in breaking through the skin. In thyroid-treated animals that are near the time for metamorphosis both forelimbs are put out with little trouble, the sinistral location of the spiracle becoming of less importance.

In a recent paper by Swingle ('23) one figure is given to show the effect of iodo-tyrosine administration in accelerating metamorphosis in pituitaryless *Rana sylvatica* tadpoles. After sixteen days of treatment the specimen illustrated has one forelimb, that one being a right forelimb. Swingle does not state when this particular right limb appeared but does say that two right forelimbs broke through as early as the eighth day, the average, however, being about twenty days. This is an interesting observation in comparison with my results after thyroid treatment; *i.e.*, 100 per cent. prior left forelimb eruption after the first two days. It seems to mean, either that iodo-tyrosine does not affect the respiratory apparatus in the same way as does thyroid extract, or that the pituitary gland plays a rôle also in influencing symmetrical development.

It now remains to discuss the normal condition and the thymus-treated condition. Three factors are considered of chief importance in determining forelimb eruption: (1) sinistral location of the spiracle; (2) relative degree of skin degeneration over the forelimb region on the two sides of the body; (3) relative size and strength of the forelimb. In partly grown thyroid-treated animals the first factor is by far the most important, as has been shown. In mature untreated tadpoles, however, this factor does not remain the all-important one. The forelimbs are now so large that the left one cannot be pushed through the spiracle without a fair amount of previous skin degeneration. Simultaneous skin degeneration occurs on both sides. The size, strength and activity of the imprisoned forelimb now becomes of

much importance. In about 30 per cent. of cases the right forelimb succeeds in overcoming the handicap of having no spiracle to come through, and breaks through by main strength before the left. In the other 70 per cent. the left limb aided by the sinistral spiracle comes through first. A majority in favor of lefthandedness is about what should be expected.

Mention should be made again of Barfurth's results. In normal *Rana fusca* tadpoles he finds that 80 per cent. put out the right forelimb first. Presumably these are like all other frog tadpoles in having sinistral spiracles although Barfurth does not refer to this feature. He believes the prevailing righthandedness of this species is accounted for by two factors: (1) earlier and greater degeneration of the skin in the right forelimb region, and (2) greater size and strength of the right limb. As these results are directly opposed to the observations of Gudernatsch, Dickerson and myself on five species of frog tadpoles, it can only be supposed that there is a species difference, and that the factors mentioned by Barfurth are strong enough to bring about a majority of righthanders in this particular species. It would be of interest to see whether in this species also the uniform lefthanded condition could be produced by thyroid treatment.

The explanation of the results after thymus treatment is somewhat more difficult and uncertain. It is probable that a shift toward righthandedness is here indicated. The ratio may be 1 : 1, although on account of the small number of animals observed in the three-limb stage, this is by no means a certainty. At any rate, the lessening of the lefthanded majority means that the asymmetrical position of the spiracle becomes of much less importance as a factor in determining the first forelimb to appear.

Desiccated thymus extract is a food rich in nutritive value, and therefore favorable to growth. Gudernatsch ('14) noted its growth-promoting effect upon tadpoles and ascribed it to the endocrine secretion of the thymus. Uhlenhuth ('17) though he combats Gudernatsch's idea as to the growth effect being due to an endocrine secretion contained in the thymus extract, states that it is a very rich and nutritious food and therefore quite favorable to growth. The writer has also observed that it is particularly favorable to limb growth. In one batch of partly-grown thymus-treated green frog tadpoles the small hind limbs

became quite red and vascular and grew rapidly, almost reminding one of the effect of thyroid extract. The writer became suspicious of the thymus extract used and had it analyzed for the presence of iodine.⁵ The analysis gave negative results. The later history of the tadpoles showed that only growth in size of the larval structures was being stimulated, and not differentiation. There was no acceleration in skin degeneration of the forelimb region, except that caused secondarily by pressure of the growing forelimb. There was likewise no reduction in the tail, but on the contrary growth. With thymus treatment limb growth appears to proceed relatively faster than the general process of body differentiation. As a result, the forelimbs enclosed beneath the skin reach a comparative size and strength, such that they become the important factor in determining the time of eruption. With increasing limb size the spiracle becomes less important since the forelimb cannot be pushed through it without complementary skin degeneration. Since size of forelimb and amount of skin degeneration on the two sides are about equal in the species under observation a more equal ratio of righthandedness to lefthandedness results.

The writer does not wish here to enter the controversy as to whether or not the thymus extract has a specific endocrine content. Its effect upon symmetry in forelimb eruption in tadpoles seems to be best explained on the grounds given above; *i.e.*, its unquestioned value as a highly nutritious and therefore growth-promoting food. It is not necessary to assume a specific endocrine effect. This much, however, may be added. Thymus gland is largely lymphoid tissue. In the light of Carrel's work showing the growth-promoting effect of leucocytic secretions or "trephones" ('24), and the confirmatory observations of Jordan and Speidel ('23) on lymphocytes in rapidly growing regions in tadpole metamorphosis, it would seem probable that growth-promoting substances (trephones) of lymphocyte origin would be present in thymus extract.

In conclusion, it may be pointed out that these observations and their interpretation, though of little importance in themselves, suggest the possibility of the following principle operating

⁵ The analysis was made by Mr. T. F. Otto, of the University of Virginia Medical School.

in any vertebrate animal in process of development: A change in the normal balance of thyroid secretion may lead to a change in the symmetrical development. Stockard ('23) has emphasized the general importance of thyroid secretion in the development of man and mammals and its part in determining the production of definite types. These results on forelimb eruption in tadpoles indicate that thyroid secretion may be of some importance also in influencing symmetrical development. A vertebrate animal, though designated as bilaterally symmetrical, is, of course, asymmetrical in many respects, *e.g.*, the visceral pattern, much of the vascular system, etc. Given an original asymmetrical condition it is possible that the thyroid may exert its effect upon the two sides of the body in a differential way.

SUMMARY.

The bilaterally symmetrical development of the frog larva is affected in a definite way by experimental hyperthyroidism. Normally in tadpole metamorphosis the left forelimb erupts first in about 70 per cent. of cases. With thyroid-accelerated metamorphosis of half-grown tadpoles practically 100 per cent. will put out the left forelimb first. Of 133 thyroid-treated animals of this kind every one protruded the left forelimb first. In the case of full-grown tadpoles already near the time of forelimb eruption, thyroid treatment may be followed during the first two days by some prior right forelimb eruptions; thereafter prior left forelimb eruption obtains. In other words, an original bias of an animal toward prior right forelimb eruption may not be changed by thyroid administration within two days.

This effect of the thyroid on symmetrical development is explicable in terms of the original asymmetrical pattern of the respiratory apparatus (*i. e.*, sinistrally located spiracle) coupled with the close anatomical relation of the forelimb to this apparatus.

Thymus treatment brings about a larger percentage of prior right forelimb eruptions, thus reducing somewhat the normal majority in favor of prior left forelimb eruption.

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